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Evaluation of survival rates of airborne microorganisms on the filter layers of commercial face masks

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Abstract

After the WHO designated COVID-19 a global pandemic, face masks have become a precious commodity worldwide. However, uncertainty remains around several details regarding face masks, including the potential for transmission of bioaerosols depending on the type of mask and secondary spread by face masks. Thus, understanding the interplay between face mask structure and harmful bioaerosols is essential for protecting public health. Here, we evaluated the microbial survival rate at each layer of commercial of filtering facepiece respirators (FFRs) and surgical masks (SMs) using bacterial bioaerosols. The penetration efficiency of bacterial particles for FFRs was lower than that for SMs; however, the microbial survival rate for all tested masks was >13%, regardless of filtration performance. Most bacterial particles survived in the filter layer (44%–77%) (e.g., the core filtering layer); the outer layer also exhibited significant survival rates (18%–29%). Most notably, survival rates were determined for the inner layers (<1% for FFRs, 3%–16% for SMs), which are in contact with the respiratory tract. Our comparisons of the permeability and survival rate of bioaerosols in each layer will contribute to bioaerosol-face mask research, while also providing information to facilitate the establishment of a mask-reuse protocol.

KEYWORDS
airborne microorganisms, bioaerosol, face mask, facepiece respirator, filter layer

Practical Implication

- Understanding the physical behavior of airborne microorganisms inside the face masks is required for protecting individual health.
- This work is designed to evaluate the microbial survival rates and their fraction of bioaerosols' activation captured in commercial face masks.
- In particular, the relative microbial survival rates at each layer were quantitatively compared.
- We confirmed that airborne microorganisms could be delivered to the inner layer, in contact with the respiratory tract while maintaining their vitality.
- The experimental results are essential to information that contributes to bioaerosol-facemask research while promoting the establishment of mask reuse protocols.

Sang Bin Jeong and Ki Joon Heo contributed equally to this work.

1 | INTRODUCTION

Since the spread of harmful airborne diseases such as coronavirus 2019 (COVID-19), the use of face masks has become strongly recommended and universalized.[1,2] Effective selection and use of face masks are essential to protect public health from the spread of airborne microorganisms (called bioaerosols).[1,3] The WHO recommends the use of respirators (e.g., N95, FFP2, or equivalent standards of face masks) during crisis situations. In particular, healthcare workers involved in the direct care of patients should use medical masks, which qualify as such after evaluation of filtration efficiency against bioaerosols (*Staphylococcus aureus*) following ASTM F2100-11.[4]

However, face masks, ubiquitous and disposable in the past, are now a scarce and precious commodity due to the global pandemic.[5] Shortages have led to additional guidelines from the Centers for Disease Control and Prevention (CDC), including the reuse of face masks intended for disposal. However, evidence to support these recommendations is lacking. In fact, previous studies have reported that the risk of bioaerosols remains, even if the face mask filters physically prevent penetration. Captured bioaerosols can maintain their vitality and multiply after ingesting the surrounding moisture and dust on the surface of filter media.[6–9] Proliferation of bioaerosols on the last (innermost) filter layer, which makes contact with the respiratory tract, can directly lead to critical secondary bioaerosol spread to users. Despite this, to our knowledge, few studies have examined how deeply bioaerosols can penetrate layers of face masks or how microbial survival differs in each layer. Therefore, an urgent need exists to evaluate the filtration performance and microbial recovery rate of each filter layer of face masks.

The objective of the present study is to determine the survivability of bioaerosols at each filter layer of commercial face masks. Physical and biological characteristics of the face masks, including filtration efficiency, pressure drop, and microbial survival rate, were evaluated using aerosolized bacterial particles. Through a better understanding of the viability of bioaerosols on filter layers, we can assess the potential for face masks to function as fomites and facilitate the development of sterilization and reuse protocols.

2 | MATERIAL AND METHODS

2.1 | Selection of face masks

Five face masks were selected; these included filtering facepiece respirators (FFRs) and surgical masks (SMs) purchased commercially in Seoul, Republic of Korea. FFRs are face masks that meet national or international standards and are predominantly required by healthcare workers.[10–14] SMs, also known as dental masks, have relatively less stringent standards compared to FFRs and have been supplied in large quantities to the market during global pandemics. In particular, one product (e.g., FFR3; The GOOD) is a public mask that was purchased on March 15, 2020, in response to continued face mask shortages. Details of face masks are presented in Table 1. All purchased face masks were multi-layer (e.g., outer-, support-, filter-, and inner-) structures as described in Figure 1A. The primary differences between FFRs and SMs included the presence of the support layer and weight per unit size. Tests were conducted by cutting a 1-inch diameter sample from the face masks and loading them onto a filter holder. Each experiment was performed in triplicate.

2.2 | Preparation of bacterial suspension

We used Gram-positive *Staphylococcus epidermidis* (KCTC 1917; Korean Collection for Type Cultures) as the focal bioaerosol. *Staphylococcus epidermidis* is not only a commensal organism of human skin, but also a bacterium commonly used in experiments using bacterial bioaerosols.[7,15] In addition, *Staphylococcus* species are used to evaluate bacterial filtration efficiency of medical face mask materials due to their harmfulness.[4,16] *Staphylococcus epidermidis* was incubated in nutrient broth (0.3% beef extraction and 0.5% peptone; Becton Dickinson) at 37°C for 24 h, until the bacterial suspensions reached an optical density of 0.6 at 600 nm. The bacterial particles were then harvested by centrifugation (5000 g, 10 min) and rinsed three times with deionized water to remove unwanted residues. Bacterial suspensions at a concentration of ~5×10^8 colony forming units (CFU)/mL were placed into a 6-jet nebulizer (Collison Nebulizer; BGI).

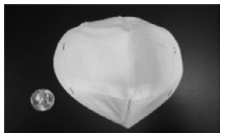
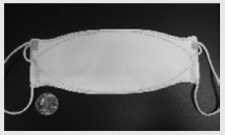
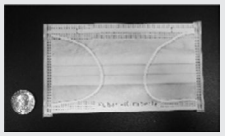
2.3 | Filtration test

A schematic diagram of the experimental setup for the filtration test is shown in Figure 1B. The filtration performance of face mask filters can be affected by airflow velocity, which simulates inhalation. First, the performance was evaluated using NaCl particles under a face velocity of 15.8 cm/s corresponding to 95 L/min, the flow rate used for face mask testing in European and Korean standards.[11,12] The flow rate, which is used to evaluate respiratory protective equipment, is relatively high because it corresponds to intense physical activity. Due to the flow conditions suitable for FFR evaluation, the filtration performance of SMs may be underestimated. The NaCl particles were generated using a 6-jet nebulizer under 1.0 psig. Before the challenge particles reached the filter medium, moisture was removed by passage through a diffusion dryer, and undesirable electrostatic charges in the particles were eliminated using a soft X-ray aerosol neutralizer (Model 4530; HCT Co.). The penetration (p) of face mask filters was calculated using the following equation:

$$p = \frac{C_{outlet}}{C_{inlet}}, \quad (1)$$

where C_{outlet} and C_{inlet} represent the particle concentrations (particles/cm^3_{air}) of the NaCl particles at the outlet and inlet of the filter holder, respectively. The size and number concentration of the particles were determined using a scanning mobility particle sizer (SMPS, model 3936; TSI Inc.), which has 108 channels ranging

TABLE 1 Specifications of tested face masks

	Type[a] (name)	Image[b]	Shape	GSM[c]	Details of face mask filter layers			
					Layer 1	Layer 2	Layer 3	Layer 4
FFR1	**N95** 3M 9010 (3M)		Cup	208 ± 10.3	Nonwoven (Outer layer)	Nonwoven (Support layer)	Nonwoven[d] (Filter layer)	Nonwoven (Inner layer)
FFR2	**KF94** Airwasher basic (LG)		Cup	193 ± 9.2	Nonwoven (Outer layer)	Nonwoven (Support layer)	Nonwoven[d] (Filter layer)	Nonwoven (Inner layer)
FFR3	**KF94** The GOOD (Jeil Pharm-aceutical Co.)		Cup	178 ± 4.3	Nonwoven (Outer layer)	Nonwoven (Support layer)	Nonwoven[d] (Filter layer)	Nonwoven (Inner layer)
SM1	**Surgical mask** Kleenguard (Yuhan-Kimberly)		Flat	64 ± 0.3	Nonwoven (Outer layer)	Nonwoven[d] (Filter layer)	Nonwoven (Inner layer)	
SM2	**Surgical mask** 3M Nexcare (3M)		Flat	73 ± 3.5	Nonwoven (Outer layer)	Nonwoven[d] (Filter layer)	Nonwoven (Inner layer)	

[a]As described on the package or according to the standard.

[b]Coin has a diameter of 25.4 mm.

[c]Grams per square meter (g/m^2).

[d]Electrostatically charged filter layer.

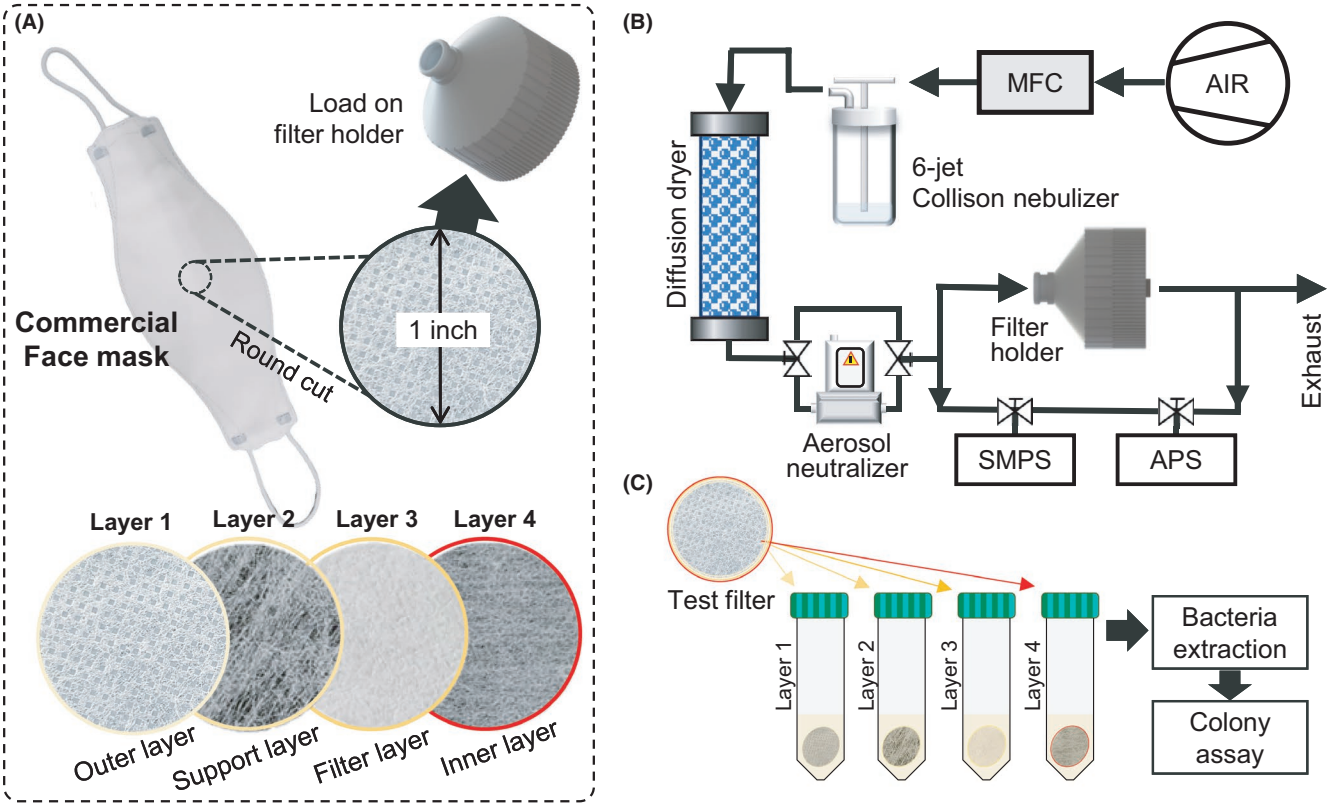


FIGURE 1 Experimental configuration of (A) the preparation of multi-layered face masks, (B) experimental setup for aerosol generation and measurement, and (C) colony analysis process for each filter layer

between 14 and 685 nm. Bacterial bioaerosols were also generated under the same nebulizer and face velocity conditions except the aerosol neutralizer, and their characteristics were measured using an aerodynamic particle sizer (APS 3321; TSI Inc.). The pressure drops across all face masks were determined using a micromanometer (MP210; KIMO Instruments) under a face velocity of 5 cm/s corresponding to 30 L/min, which is the standard airflow rate of aforesaid European and Korean face mask test standards. In addition, the filter quality factor (QF) was used to compare performances, as follows:

$$QF = \frac{-\ln(p)}{\Delta P}, \tag{2}$$

where p is the penetration and ΔP is the pressure drop. QF is an indicator of pressure drop and filtration efficiency comparisons under the same face velocity conditions; the higher the value, the more efficient the filter. Because the circular samples punched from each face mask were sealed using a filter holder, the leakage rate was not a consideration.

2.4 | Microbial survival rate

Microbial survival rates were calculated using the ratio of active proportions of bacteria from tested face masks. The *S. epidermidis* particles were deposited onto the face masks for 10 min using the nebulizer. Then, the filters were exposed to room temperature biosafety cabinet for 10 min, to treat the damage caused by desiccation effects (e.g., natural decay) equally.[17] The filter samples were carefully separated into individual layers using sterilized forceps. Each filter layer was cut into pieces using sterilized medical scissors and immersed in a 10-mL suspension ($V_{extraction}$) of phosphate-buffered saline (PBS) containing 0.01% Tween 80 (Figure 1C). The suspension containing the bacteria-deposited layer was run through an extraction process (5 and 3 min of vortex and sonication, respectively) to transfer the bacterial particles from the filter layers to the PBS suspension. The resulting bacterial suspension was serially diluted onto nutrient agar plates (0.3% beef extraction, 0.5% peptone, and 15% agar; Becton Dickinson) and incubated at 37°C for 24 h. Colony growth on the plates was enumerated after incubation, and microbial survival rates were calculated as follows:

$$\text{Filtration efficiency}\,(\eta) = 1 - p \tag{3}$$

$$CFU_{total} = \sum CFU_{layer,n} \tag{4}$$

$$N_{filter} = C_{inlet} \cdot Q_{sampling} \cdot \eta \cdot \frac{\xi_{extraction}}{V_{extraction}} \tag{5}$$

$$\text{Microbial survival rate}\,(\%) = \left(\frac{CFU_{total}}{N_{filter}}\right) \times 100 \tag{6}$$

where CFU_{layer} is the concentration (CFU/mL) of live bacteria recovered from each filter layer and CFU_{total} is the sum of all CFU_{layer} in a single group. N_{filter} is the total concentration of bacterial particles in the extract-suspension plated on the agar (particles/mL), and $Q_{sampling}$ is the total airflow sampling volume. $\xi_{extraction}$ is the physical extraction of bacteria at each filter layer, defined as the ratio of the number of particles transferred from the filter to the extraction suspension to the number of particles removed from airflow using the filter. $\xi_{extraction}$ for all filter layers was evaluated using the comparable method as >95%, as proposed by Wang et al (2001)[18].

2.5 | Statistical analyses

Normal distributions of results were verified using Shapiro-Wilk and Kolmogorov-Smirnov tests. Analysis of variance (ANOVA) and Kruskal-Wallis tests were conducted to compare normally distributed and non-normally distributed data, respectively, to compare penetration, pressure drop, and microbial survival rate. Correlation coefficients, linear regressions, and *t* tests of experimental results were analyzed using SPSS software (ver. 21; SPSS Inc.).

3 | RESULTS AND DISCUSSION

3.1 | Comparison of penetration and pressure drop

To quantify the performance of tested face masks, filtration performance was evaluated using NaCl particles. The NaCl particles were log-normally distributed with a mode diameter of 26.7 ± 1.36 nm, a geometric mean diameter (GMD) of 37.6 ± 1.32 nm, a geometric standard deviation (GSD) of 1.92 ± 0.122, and a total concentration of 1.19 ± 0.696 × 10^7 particles/cm^3_{air} (Figure 2). NaCl particles were used not only because they are the challenge aerosols used in face mask test protocols in European and Korean standards, but also because they have a universal size distribution that includes the sizes of all hazardous airborne substances, such as viruses and bacteria.[3] Therefore, NaCl particles were used as a benchmark for measuring the performance of commercial face masks.

The filtration characteristics of commercial face masks using NaCl particles are presented in Figure 3A. The average penetration of FFRs was 2.6 ± 0.64%, meeting the standard value defined by the Korean government (<6%), and differences among the three FFR masks were not statistically significant (*t*-test: $p > 0.05$). However, the SMs exhibited significant performance deviation ($p < 0.01$) compared to FFRs, likely due to the lack of legally enforced certification parameters for the direct management of SM filtration performance. In addition, SMs generally exhibited a high penetration rate (>60%). This difference in performance between FFRs and SMs is due to both the number of layers and the basis weight of the face masks. FFRs consist of four filtration layers, and the basis weight is higher than 170 g/m^2_{filter}; by contrast, SMs consist of only three filtration layers and the basis weight is only 60–70 g/m^2_{filter}.

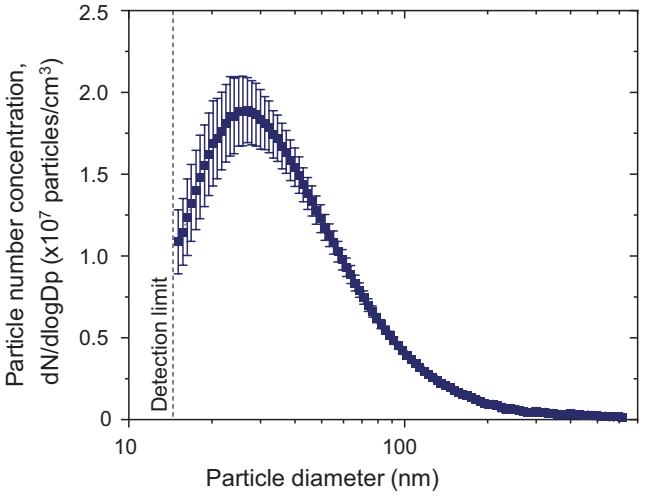


FIGURE 2 Size distribution of NaCl particles. Error bars indicate standard deviation ($n = 3$)

The compositional differences in filter characteristics were demonstrated by measurements of pressure drop (Figure 3B). Pressure drops in the FFRs were all higher than 60 Pa, with a maximum value of 106 ± 4.2 Pa for FFR2. The standards for FFRs (e.g., FFP2 in Europe and KF94 in Korea Standards) are based on less than 70 Pa of pressure drop under 30 L/min. All FFR standards were satisfied for penetration; however, FFR1 and FFR2 exhibited pressure drops exceeding 70 Pa, which resulted from differences in testing methods. While the authorized methods are to conduct tests by placing the face masks directly on a dummy head, our tests used a filter holder without leakage. In addition, FFRs and SMs serve different purposes and uses. SMs are respiratory personal protective equipment (PPE) used to protect the users' face from large infectious droplets (e.g., >5 μm), such as water, blood, and saliva. On the other hand, FFRs are respiratory PPE made to protect against infectious airborne particles (e.g., <5 μm); thus, they are designed to be heavier and more demanding than SMs. Several studies have shown that FFRs are effective at blocking micron-scale bioaerosols, but the use of SMs for the same purpose would be concerning.[19–21] Our results using NaCl particles were consistent with these previous results.

Due to a trade-off between filtration performance and pressure drop, optimization is critical for the selection of face masks. The QF is widely used to determine whether an air filter is efficient (Figure 3C). The QFs of FFRs, which have lower penetration, were higher than the values for SMs. The face mask with the least pressure drop (i.e., easy to breathe) while blocking challenge aerosols most effectively was FFR3. This result indicates that the user should select commercial face masks by taking into account not only the filtering efficiency, but also the pressure drop. In particular, it is essential for heavy-duty workers, the elderly, and children to choose face masks that are effective and allow for easy breathing.

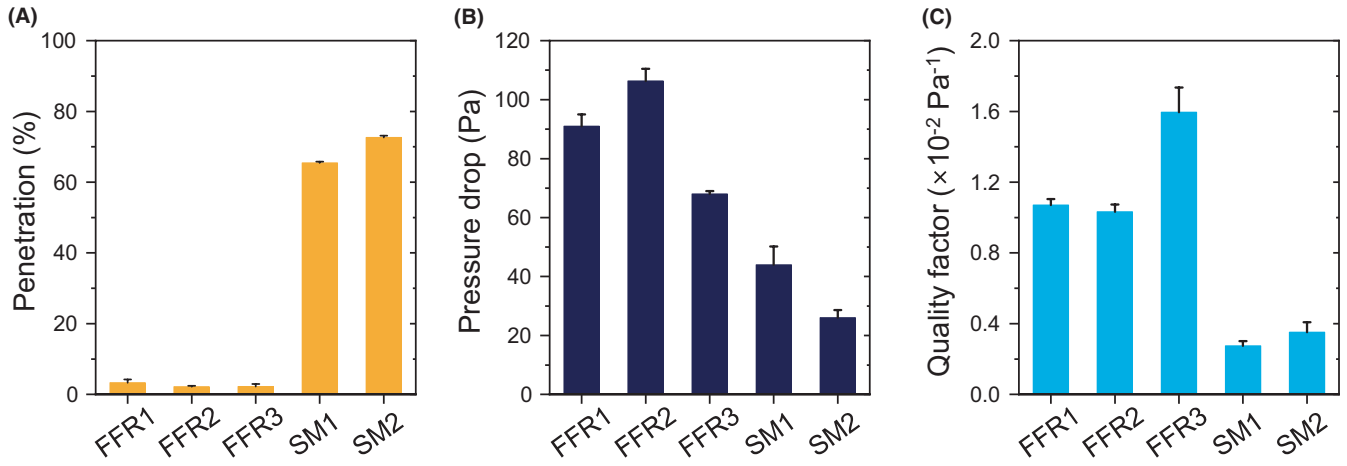


FIGURE 3 (A) Filtration performance against NaCl particles, (B) pressure drop, and (C) filter quality factor of face masks under relevant face velocity conditions. Error bars indicate standard deviation (n = 3)

3.2 | Microbial penetration and survival rate

The physical characteristics of aerosolized bacterial particles measured by APS are described in Figure 4A. The size distribution of *S. epidermidis* was log-normally distributed with a mode (peak) diameter of 0.9 ± 0.01 μm, a GMD of 0.9 ± 0.01 μm, a GSD of 1.2 ± 0.01, and a total concentration of 1.2 ± 0.06 × 10^3 particles/cm^3_{air}. The filtration efficiencies against bacterial bioaerosols are described in Figure 4B. NaCl and bioaerosol filtration performances of the FFRs did not significantly differ (>94%, p > 0.05); however, the filtration efficiency of SM1 increased from 35 ± 0.4 to 94 ± 1.5%, and that of SM2 increased from 28 ± 0.5 to 46 ± 9.3% as the challenge particles were changed from NaCl to bioaerosols.

The penetration of bioaerosols should not be considered the same as that of ordinary particles. Even in small quantities, bioaerosols can cause severe disease via their biological properties and proliferation. Accordingly, various studies have been conducted on the filtration performance of face masks against viral or bacterial bioaerosols. For example, Jeong et al.[7] reported that FFRs exhibited penetration against bacterial bioaerosols of ~1%, while SM had penetration levels of ~20%. The penetration of FFR against MS2 viral bioaerosols (nominal virion size of 0.02–0.09 μm) was >95% under similar air flow conditions of 85 L/min.[3] In addition, several studies have indicated that SMs may not be adequate to prevent direct exposure to submicron-bioaerosols.[6,19,22] This unreliable filtration performance of SMs indicates that more attention should be paid to the selection of face masks.

Figure 4C presents the microbial survival rates, as defined by Eq. 6. The highest and lowest survival rates were 18.5 ± 0.31% (FFR1) and 13.8 ± 0.19% (FFR2), respectively. The correlation between filtration efficiency and survival rate was insignificant. Regardless of the filtration efficiency against bacterial particles, the survival rates from all mask filters were >13%. Although FFRs have excellent filtration performance, the observed microbial survival rates suggest the possibility of masks serving as fomites of secondary damage. SMs, on the other hand, pose risks from both

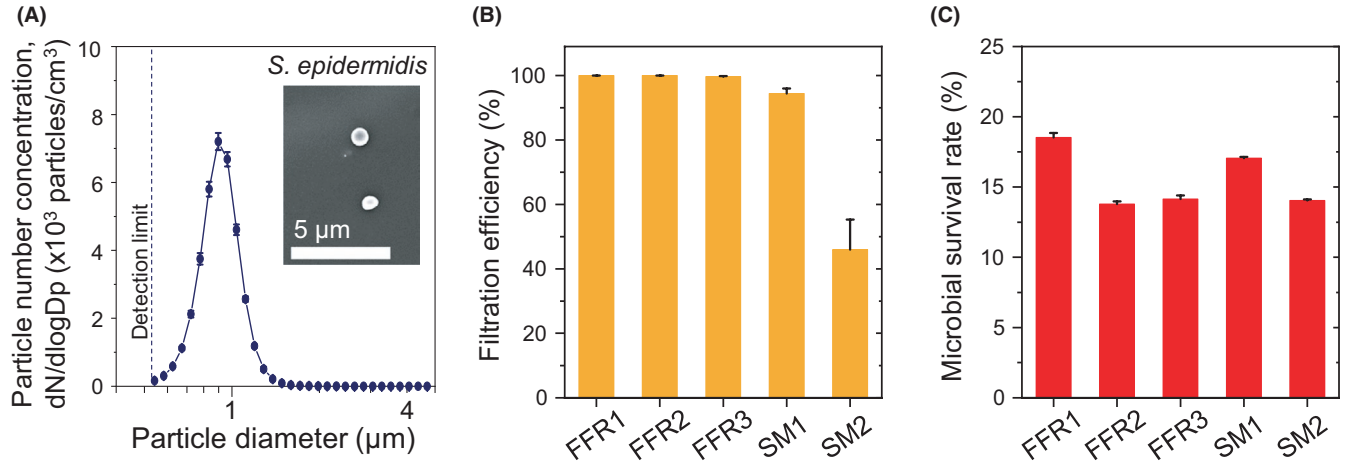


FIGURE 4 (A) Size distribution and SEM image of *Staphylococcus epidermidis* bioaerosols. (B) Filtration efficiency against bacterial bioaerosols. (C) Microbial survival rate of tested face masks. Error bars indicate standard deviation (n = 3)

the penetration of bioaerosols and from microbial survival. While survival rates in the present study were evaluated using only one Gram-positive bacterium, *S. epidermidis*, previous research has demonstrated that various filtered bioaerosols are viable on face mask filter surfaces.[6,7,23]

The fractions of captured and activated bacterial particles (shown in red in Figure 5) were evaluated by multiplying the filtration efficiency (e.g., the amount of bacterial particles deposited on the filter) by the microbial survival rate. FFR1 accounted for the largest bacterial activation fraction (18.5%), while this value was low in SM2 (6.5%). In contrast to FFRs, only a small amount of bacterial particles was captured in SM2. The activation fraction of bacteria differed among products, but the differences were not statistically significant. In general, airborne microorganisms may lose their vitality and culturability during aerosolization due to the physical stress and exposure to a relatively harsh environment. In addition, bacterial particles may be affected by desiccation (e.g., natural decay) on the surface of the air filter. The natural decay may cause Gram-positive bacterial particles to become 72% and 92% inactive in 10 and 60 min, respectively, on the filter surface.[24] Although many of the bacteria had been inactivated, these results highlight the tenacious vitality of bioaerosols. Even if they exist in only a small fraction, biological substances with ample space for proliferation require continual vigilance.

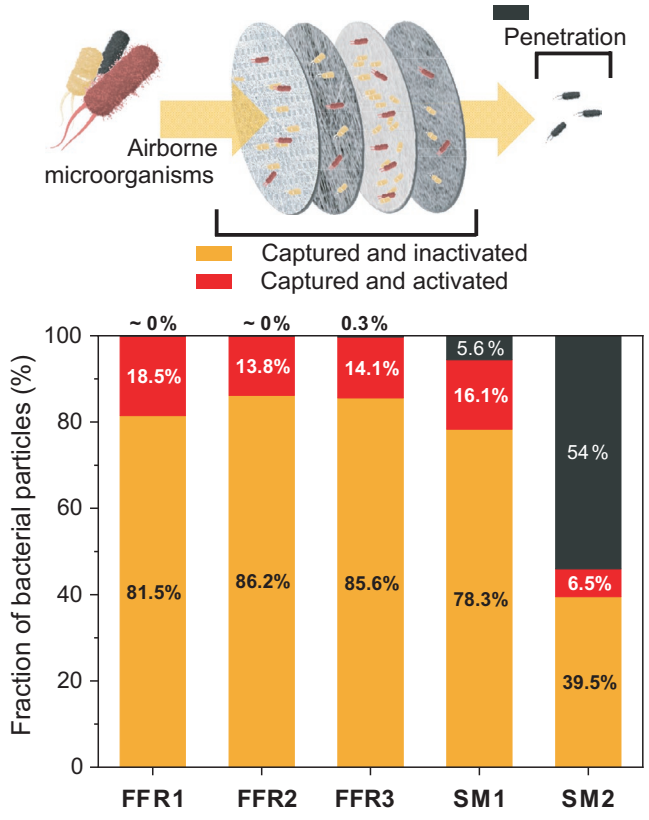


FIGURE 5 Fractions of bacterial bioaerosols including penetration, captured bacteria, and captured but activated bacteria

3.3 | Microbial survival rates at each layer

After the intact face mask samples filtered the bacterial bioaerosols, the relative microbial survival rates in each layer were evaluated (Figure 6). Overall, captured and activated bacteria existed at high rates in the filter layers (e.g., Layer 3 and Layer 2 for FFRs and SMs, respectively), consistent with existing research that most particulate pollutants are captured by the filter layers. The relative microbial survival rates of the inner layers were less than 1% in FFRs, but were high in SM1 and SM2 at 3.2% and 16.7%, respectively. In addition, the survival rate on the inner layers tended to be similar to the penetration rate of the face mask samples.

The outer layer, which is used to protect mask structures (rather than filtering particles), exhibited significantly high rates of microbial survival. Because most airborne bacteria adhere to relatively large particulate matter rather than to themselves,[25] field tests are expected to show more bacterial recovery from the outer layer. Therefore, it is important not to touch the outer layer with bare hands as much as possible, and care should be taken when disposing of used face masks. In the case of FFR2, the survival rates in Layers 1 and 2 were relatively high compared to the other FFRs, which is likely related to the pressure drop of the sample. FFR2 was the face mask sample with the highest pressure drop, and local atmospheric stagnation due to the high pressure drop at the front end of the filter layer may have occurred. As a result, many bacterial particles could adhere to Layer 2 with little filtration efficiency, leading to a relatively high survival rate distribution.

To intuitively verify the distribution of bacterial particles in the filter layers, scanning electron microscopy (SEM) analysis was conducted using FFR1 and SM1 face masks, both of which had relatively high microbial survival rates. FFR1 and SM1 were exposed to bacterial particles under conditions of 15.8 cm/s of face velocity and then separated by layer (Figure 7). The fiber diameters of the outer and inner layers of both samples were similar (18–21 μm), and no

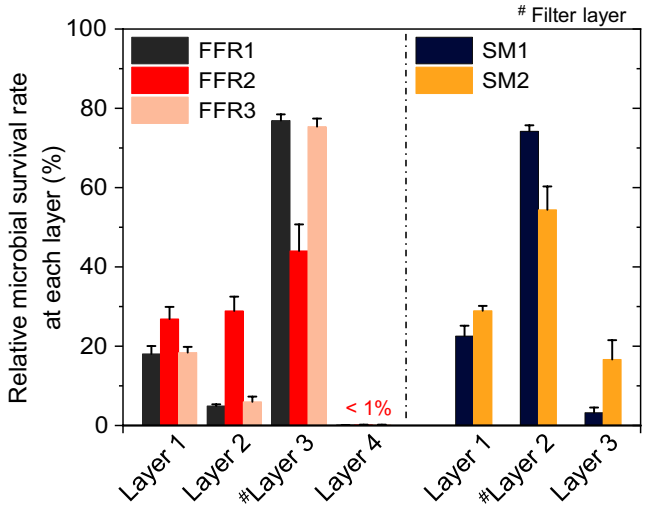


FIGURE 6 Relative microbial survival rate for bacterial particles at each face mask filter layer. Error bars indicate standard deviation (n = 3)

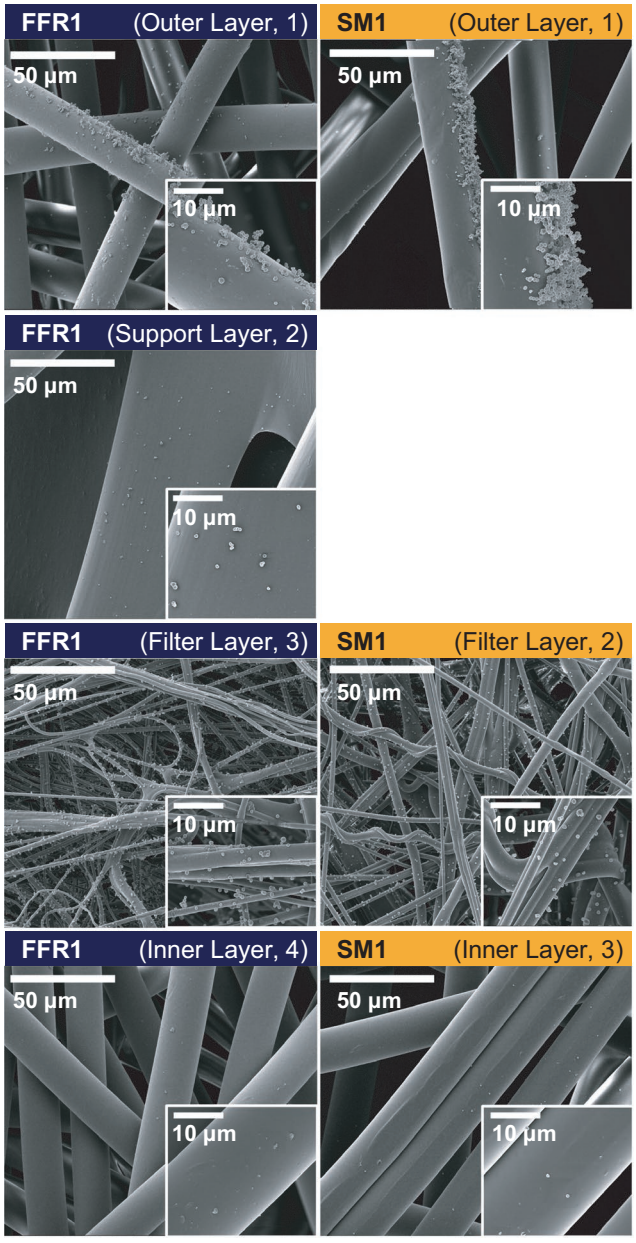


FIGURE 7 SEM images of layers of FFR1 and SM1 with captured bacterial particles

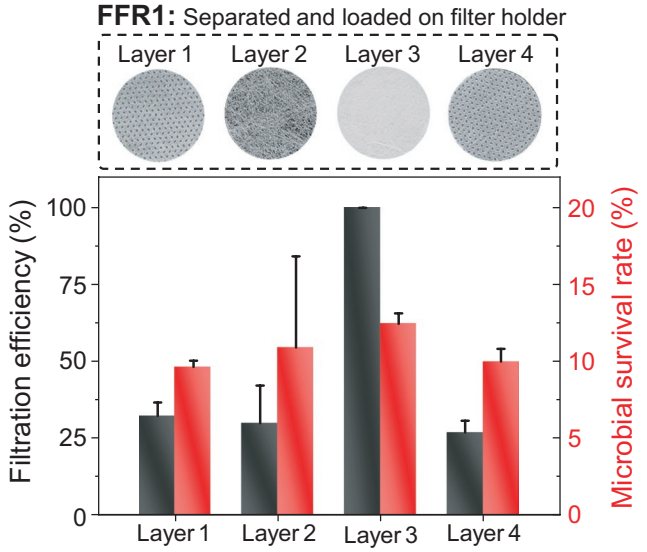


FIGURE 8 Independent filtration efficiency and microbial survival rate of each layer from FFR1. Error bars indicate standard deviation (n = 9)

significant structural differences of the filter layers were identified via SEM analysis. Most bacterial particles were captured in both filter layers, but a considerable number were observed in the outer layers. In addition, while few bacterial particles were observed in the inner layer of FFR1, particles were identified in the inner layer of SM1, which is consistent with the relative microbial survival rates at each layer described above.

To determine whether the components of each mask layer affect bacterial vitality, the filter layers of FFR1 were separated independently and loaded onto the filter holder (Figure 8). The filtration efficiency and survival rate of a single layer were evaluated using the same test protocols described above. The filtration efficiency was highest in layer 3 (~99%), which had the thinnest filter diameter and highest electrostatic charge. The other layers exhibited efficiencies <33%. The highest survival rate was 12.4% for layer 3 and ~10% for the other layers. The correlation between filtration efficiency and survival rate was not significantly different among layers ($p > 0.05$). We concluded that while the microbial survival rate in a single layer was relatively small, the structural or material differences of each layer have little effect on survival rates. As described above, certain portions of the particles captured by the filter always survived.

The key result of our testing is that bioaerosols can be delivered to the inner layer of face masks while maintaining their vitality. The inner layer is in direct contact with the respiratory tract and is therefore always exposed to moisture during breathing. In addition, due to the body temperature of the user, the inner layers would serve as suitable environments for microorganism proliferation, thus facilitating secondary damage.

Bacterial survival results in various filter layers suggest that the use of antimicrobial properties on filters or the sterilization process are necessary to wear face masks safely. Antimicrobial air filters deactivate harmful bioaerosols on the surface of fibers. Accordingly, antimicrobial filter technology that employs various antimicrobial components including inorganic and organic materials has been actively developed.[15,17,26] When sterilizing face masks for reuse, it is important to inactivate the target bioaerosols while not affecting the structure or performance of the filters. During this process, care should be taken not to disrupt electrostatic charge, which plays an important role in filter quality. Ultraviolet germicidal irradiation (UVGI) would serve such a purpose and is one of the most recommended methods for the reuse of face masks during global pandemics.[27,28] Studies have shown that UVGI is effective in controlling bioaerosols while maintaining the filtration efficiency of the respirator.[29,30]

The limitations of this study should be addressed in future research. This study only dealt with one Gram-positive bacterium, *S. epidermidis*. Although this bacterium has traditionally been used in various bioaerosol studies, it does not represent all microorganisms, as bioaerosols encompass bacteria, fungi, and viruses of various sizes with unique biological attributes. The performance of face masks can vary depending on the biological nature of microorganisms, which differ in survival time and proliferative potential. Therefore, face mask research should consider various biological substances, including pathogenic bacteria and viruses. In addition, the fit performance of face masks was not considered. Since FFRs include more considerations for fit performance than do SMs, the penetration of bioaerosols may be enhanced in SMs when used in practice. Research considering real-world use would be more reliable and could produce universally applicable results.

4 | CONCLUSION

Our results indicate that wearing face masks cannot completely prevent the threat of bioaerosols. Commercial FFRs were effective in physically blocking bioaerosols (>99%), but SMs were not. The microbial survival rates were greater than 13% in all face masks, regardless of filtration performance. In particular, the total survival rate and permeability of particles were carefully compared by layer, which goes a step further than existing bioaerosol-respirator viability studies. The viability of microorganisms was confirmed in the inner layer of face masks. Most notably, SMs, which exhibited lower filtration efficiency against bacterial particles, showed a higher portion of microbial viability in the inner layer. Because the inner layer of face masks is in contact with the respiratory tract, it will be important to be mindful of potential bioaerosol proliferation on this surface. Therefore, certain occupational groups exposed to biological threats must use FFRs and are encouraged to use antimicrobial filters or undergo mask sterilization processes to fully prevent secondary damage.

CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

Sang Bin Jeong and Ki Joon Heo involved in conceptualization, data curation, writing-original draft, investigation, writing-review and editing. Hyun Sik Ko involved in data curation, formal analysis, and investigation. Jae Pyoung Ahn involved in funding acquisition and validation. Seung-Bok Lee involved in supervision, project administration, funding acquisition, and validation. Jae Hee Jung involved in supervision, project administration, methodology, validation, writing-review and editing.

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